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Original article

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Methane production from organic acids obtained by supercritical water treatment
of Japanese beech

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Key words: Japanese beech; supercritical water; pressure; organic acid; methane
fermentation

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Abstract

Japanese beech (*Fagus crenata*) wood was treated in supercritical water at 380 °C / 30 MPa and 100 MPa. The hydrolysate (water-soluble portion) was found to contain the fragmented and dehydrated compounds of sugars and organic acids.

5 Although organic acids are expected to be utilized for methane fermentation, the effects of the fragmented and dehydrated compounds of sugars on the methane production are not known. The objective of this study is, therefore, to elucidate the potential of supercritical water treatment as a new pretreatment for methane production by evaluating the methane fermentability of the hydrolysate. From the
10 methane fermentation tests for those model compounds with digested sludge, it is found that methane was produced not from the fragmented and dehydrated compounds but from the organic acids. The yield of methane from the hydrolysate obtained at 380 °C/ 30 MPa was higher than that from the hydrolysate obtained at 380 °C/ 100 MPa. This is due that the former contains much organic aids than the
15 latter. The maximum yield of methane was seven times greater compared to the untreated wood, indicating that the supercritical water treatment is effective for enhancing the productivity of methane from wood.

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Introduction

With the possibility of depletion of the world's petroleum supply, biomass has attracted attention as an alternative energy resource. The lignocellulosics is one of the major biomass as renewable resources.¹ Although a large amount of lignocellulosics such as wood, for example, is used as materials for building construction, those wastes are not utilized effectively. To accelerate the utilization of such waste lignocellulosics, the conversion technologies for them are thought to be indispensable. Among various technologies, methane fermentation process is expected to be one of the promising technologies. However, due to the nature and structure of lignocellulosics, it is difficult to convert it to methane by anaerobic fermentation. Lignocellulosics, the cell wall structural material, is composed of cellulose and hemicellulose encrusted with lignin. Such a complex structure of lignocellulosics prevents the enzymatic accessibility of the anaerobic microorganisms to the cell wall components. This resistance to biodegradation has led to develop the pretreatment methods to enhance the biodegradability of lignocellulosics.²⁻⁵

Amirta et al. studied pretreatment using white rot fungus for methane fermentation of Japanese cedar wood.⁴ They reported that fungal pretreatments affect as delignification to enhance the methane fermentation. Take et al. also examined the production of methane gas from Japanese cedar chips pretreated by various delignification methods such as refiner treatment, steaming treatment, fungal treatment and steam explosion.⁵ As a consequence, steam explosion was most efficient pretreatment method for obtaining the high methane yield. Although methane gas was not evolved from untreated wood chips, 180 ml of methane gas was obtained from 1 g of wood chips after steam explosion. They indicated that increasing surface area of wood and delignification is a major factor to enhance the methane fermentation.

On the other hand, supercritical water treatment ($> 374\text{ }^{\circ}\text{C}$, $> 22.1\text{ MPa}$) has been considered to be an attractive alternative technology. It is reported that various sugars can be obtained from cellulose^{6,7} or lignocellulosics^{8,9} as treated in supercritical water. Furthermore, when the treatment is prolonged, some organic acids can be also obtained as well as dehydrated and fragmented products of sugars.^{10,11} These organic acids are reported to be mainly derived from cellulose and hemicellulose in Japanese beech (*Fagus crenata*), but partly from lignin propyl side-chains as relatively prolonged treatment was applied.¹⁰ Since organic acids can be used as good substrates for methane production by microorganisms,¹²⁻¹⁴ supercritical water treatment is expected as a pretreatment for methane fermentation. As mentioned above, some research has conducted previously for enhancing the methane production from wood. The objectives of those studies are to remove lignin and to increase surface area of wood for improving the accessibility of microorganisms to wood. Our proposed pretreatment with supercritical water, on the other hand, aims to produce organic acids from wood, which is new idea and completely different from those previous methods. However, not only organic acids but also the dehydrated and fragmented products of sugars and the lignin-derived products are produced simultaneously by supercritical water treatment of wood. The effects of these products on the methane production are not known, which means it is not possible to estimate the productivity of methane only from the amount of organic acids obtained. The objective of this study is, therefore, to evaluate the methane fermentability of the hydrolysate, which contains various compounds such as organic acids, the dehydrated and fragmented products of sugars and the lignin-derived products obtained by supercritical water treatment of Japanese beech. To optimize methane gas evolved, supercritical water treatments ($380\text{ }^{\circ}\text{C}$ / $30 - 100\text{ MPa}$) in various conditions were conducted as pretreatments. Methane fermentability of each model compound obtained from Japanese beech was also investigated.

Materials and methods

Samples and chemicals

5 Wood flour passed with 80 mesh from Japanese beech (*Fagus crenata*) was used as a lignocellulosics sample. As chemical reagents, dihydroxyacetone (purity: 95%), glycolaldehyde (purity: 95%), furfural (purity: 98%), 5-hydroxymethylfurfural (5-HMF) (purity: 95%), formic acid (purity: 98%), glycolic acid (purity: 98%), acetic acid (purity: 99%) and lactic acid (purity: 91%)
10 were purchased from Nakalai Tesque, Inc., Kyoto, Japan. For the supercritical water treatment, water in high performance liquid chromatography (HPLC) grade was used. Prior to the supercritical water treatment, Japanese beech was Soxhlet-extracted by mixture of ethanol and benzene (1: 2 in volume) for 12 h.

15 Supercritical water treatment

 Treatments were conducted by using batch-type system as described in previous study.⁷ A sample was slurried at 3 wt% by adjusting water. To control the reaction pressure, this slurry was fed in prescribed volume to a 5 ml reaction vessel made of Inconel-625 as shown in Table 1. A void volume of reaction vessel
20 was charged by nitrogen. The reaction vessel was heated by immersing it into the molten tin bath preheated 500 °C until the temperature in the reaction vessel was reached up to 380 °C. At adequate reaction time, the vessel was immersed in the water bath to quench the reaction. During this treatment, the temperature and the pressure in the reaction vessel were monitored by a thermocouple and pressure
25 gauge attached to the reaction vessel, respectively. In this batch-type system, temperature and pressure are both changeable so that the treatment condition was defined by maximum temperature and maximum pressure during the experiment. The detail of reaction temperature and pressure profiles was presented in our

previous report.⁷

The obtained reaction mixture was filtrated with 0.2 μm membrane filter to separate the water-soluble portion (hydrolysates) from the water-insoluble residue. The water-insoluble residue was dried at 105 °C for 24 h and weighed to
5 obtain yield. The yield was calculated by the following equation:

$$\text{Yield (\%)} = W_I / W_0 \times 100$$

where W_0 is the amount of charged sample, and W_I is the weight of
10 water-insoluble residue.

Methane fermentation

To evaluate the methane fermentability of the hydrolysates from beech wood as treated in supercritical water, methane fermentation was carried out by
15 using the apparatus as shown in Figure 1. Cultures were conducted in a 200 ml vial containing 50 ml of digested sludge (Suspended solids: 2.0 %) from Kyoto Municipal Bio-Gasification Demonstration Plant and 5 ml of the hydrolysates. The fermentation vials were purged with argon gas and incubated at 50 °C for 35 days. Methane produced was collected in a measuring cylinder. As a comparison, 150
20 mg untreated beech wood and 4.9 ml of water were fermented in 50 ml digested sludge in a similar manner. Furthermore, formic acid, glycolic acid, acetic acid, lactic acid, dihydroxyacetone, glycolaldehyde furfural and 5-HMF which were obtained commercially as described above were also fermented in the same system. 100 mg of these compounds was enclosed with 4.9 ml water in vial containing 50
25 ml of digested sludge to start fermentation. In addition, gas production from vials containing only the digested sludge without wood flour was measured as a control.

Analytical methods

The obtained water-soluble portions were analyzed by capillary electrophoresis (CE) and HPLC. In order to quantify the organic acids, CE analysis was conducted by HP3D CE systems (Agilent Technologies). The fused-silica capillary (75 μ m in inner diameter, 104 cm in total length, 95.5 cm in effective length) from Agilent technologies was used. The indirect UV detection at 270 nm was performed using the 2,6-pyridinedicarboxylic acid buffer for organic acid analysis.

For the determining lignin-derived compounds, HPLC analysis was conducted by Shimadzu, LC-10A equipped with a STR ODS-II column (Shinwa Chem. Ind. Co.) and an ultraviolet detector (Shimadzu, SPD-10A) set at 280nm. CH₃OH/H₂O (20/80→100/0, 0-60min) was used as mobile phase at a flow rate of 1.0ml/min. The column oven temperature was set at 40°C.

For quantification of various products except for the organic acids and lignin-derived compounds, another HPLC analysis was conducted by Shimadzu LC-10A equipped with Shodex Sugar KS-801 (Showa Denko) column and a refractive index detector (Shimadzu, RID-10A) using water as mobile phase at a flow-rate 1.0 ml/min. The column oven temperature was set at 80 °C.

To determine methane gas evolved during the methane fermentation, gas chromatography (GC) analysis was conducted by a Shimadzu GC-14A equipped with a thermal conductivity detector (TCD). Separation was performed on a stainless column, SHINCARBON ST (Shinwa chemical industries) using argon as a carrier gas at a flow-rate of 30 ml/min. Temperatures of the column, injector and detector were set at 40°C.

Results and discussion

Table 2 shows total content of water-soluble portion (hydrolysates) and water-insoluble residue from Japanese beech wood as treated in various

conditions of supercritical water. The yield of the water-insoluble residue was around 20 % in any reaction conditions except for the reaction at 380 °C/ 30 MPa/ 60s. On the other hand, in the water-soluble portion, 5-HMF and furfural as dehydrated products, dihydroxyacetone and glycolaldehyde as fragmented products and formic acid, acetic acid, glycolic acid and lactic acid as organic acids were identified by HPLC and CE analyses.

The dehydrated products were hardly produced under the reaction of 30 MPa/ 380 °C, while that in 100 MPa/ 380 °C was about 8 % for 7 s treatment. However, the yield of the fragmented products at the condition of 30 MPa / 380 °C was higher than that of 100 MPa/ 380 °C for 7 s treatment. These results are in good agreement with our previous studies in which the dehydrated products are preferentially produced at high pressure with short reaction time while much fragmented products are obtained at low pressure with short reaction time.^{10,11} At lower pressure of 30 MPa, the yields of total organic acids were over 20 %. The maximal production of total organic acids can be attained at 30 MPa for 60 s treatment with 23.1 % in yield. On the other hand, at higher pressure of 100 MPa, the yields of the total organic acids were around 16 %. As reported previously,¹⁰ the fragmented products are preferentially produced in lower pressure treatment than 100 MPa. It was reported that organic acids can be easily produced from the fragmented products rather than the dehydrated products.¹¹ Therefore, high yield of organic acids were obtained at 30 MPa treatment in supercritical water compared to high pressure treatment such as 100 MPa.

To examine the fermentability of various compounds obtained in hydrolysates from beech wood as treated in supercritical water, the organic acids (formic acid, acetic acid, glycolic acid and lactic acid), the dehydrated products (5-HMF and furfural) and the fragmented products (glycolaldehyde and dihydroxyacetone) were fermented with the digested sludge.

Figure 2 shows the comparison of the methane production among various

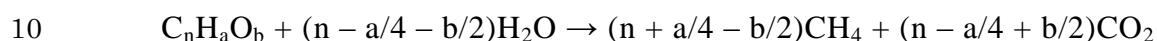
organic acids such as (a) acetic acid, (b) glycolic acid, (c) formic acid and (d) lactic acid. To evaluate the conversion efficiency of these organic acids to methane, left axis shows the mol number of methane production par 1mol of each organic acid, and right axis shows the volume of the actually produced methane. As a result, the yield of methane from acetic acid rapidly increased for 8 days incubation, and subsequently remained constant. The yield of methane from lactic acid increased for 10 days. After 10 days incubation, that was slightly decreased. On the other hand, the yields of methane evolved from glycolic acid and formic acid gradually increased as the incubation time extended. The 11.5 ml, 13.4 ml, 29.6 ml and 31.9 ml of maximum methane production were attained during 30 days incubation from formic acid, glycolic acid, acetic acid and lactic acid, respectively. It means that 0.22 mol, 0.42 mol, 0.75 mol and 1.11 mol of methane were evolved from 1 mol of each organic acid, respectively. All organic acids studied were found to be converted to methane by the digested sludge, although the duration for the fermentation was varied. On the other hand, it is also found that methane from 5-HMF, furfural, glycolaldehyde and dihydroxyacetone were hardly produced in 30 days incubation. (data not shown)

This digested sludge, which has such activity for methane production from various organic acids as shown in Figure 2, was applied in the following fermentation experiments. Methane evolved from water-soluble portion (hydrolysates) of beech wood as treated for various reaction times in supercritical water (a) 380 °C/ 30 MPa and (b) 380 °C/ 100 MPa were investigated as shown in Figure 3. For comparisons, the results of the untreated wood and only digested sludge without any substrates are also included in Figure 3. Methane production increased with the increase in the incubation time for 35 days. The maximum methane evolved was 0.66×10^{-3} mol (15.8 ml) at 30 MPa/ 380 °C for 240 s (Figure 3(a)). This value was 7 times higher than that from untreated wood. Furthermore, a higher methane production was achieved at 30 MPa (Figure 3(a))

than at 100 MPa (Figure 3(b)). As shown in Figure 2, methane can be produced not from the dehydrated and fragmented products but from organic acids. Thus, the results in Figure 3 are possibly due to the difference in the yields of organic acids from beech wood as treated in supercritical water treatment as shown in Table 2.

5 Therefore, the relationship of methane evolved and organic acids obtained by supercritical water was investigated.

For the methane fermentation of organic compounds, a reaction formula as shown below was proposed by Buswell and Muller.¹⁴



The yields of methane from the organic acids in the hydrolysate were calculated by this reaction formula, based on the yield of the organic acids shown in Table 2. The percentages of methane actual evolved in Figure 2 to the calculated yield of methane are shown in Table 3. At the reaction conditions of 380 °C/ 100 MPa for 7 s, the percentage of actual methane evolved was 37 % of the calculated value. However, except for this reaction condition, all the percentages of actual methane evolved to the calculated value from organic acids were over 90 %. These results in Figure 3 and Table 3 show that methane can be produced mainly from organic acids in the hydrolysates.

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Serra-Alvarez and Lettinga reported that some lignin-derived compounds inhibited methane fermentation.¹⁵ In our previous study, it was indicated that the large part of lignin-derived compounds were obtained as water-insoluble residue, however, a trace amount of these compounds was present in water-soluble portion from beech wood as treated in supercritical water.^{9,16} Figure 4 shows the HPLC chromatograms of the water-soluble portion from Japanese beech as treated in the condition of 380 °C/ 100 MPa. Some lignin-derived compounds such as vanillin, acetovanillon guaiacol, coniferylalcohol and methylguaiacol can be determined.

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These results in Table 3 and Figure 4 indicate that although some lignin-derived compounds exist in the hydrolysates, their inhibitory effects on the methane fermentation are not significant. This may be because the hydrolysates do not contain so much lignin-derived compounds that can prevent any methane productions. This fermentability is preferable because some treatments are not required to improve the fermentability of the hydrolysate. However, as shown in Figure 4, the height of the peaks for various lignin-derived compounds in HPLC chromatograms is varied with the reaction time, indicating the composition of the lignin-derived compounds is different in each water-soluble portion. This difference may affect on the methane production and cause low methane productivity at the condition of 380 °C/ 100 MPa/ 7 s as shown in Table 3.

Consequently, it was indicated that methane was mainly produced not from the dehydrated products and fragmented products, but from the organic acids in the hydrolysates from beech wood as treated in supercritical water (380 °C). Significant inhibition for methane production could not be observed. Therefore, supercritical water treatment at 30 MPa was more appropriate than at 100 MPa as pretreatment for enhancing methane production from wood because the higher yields of the organic acids can be attained at former reaction condition. For future industrial application, much larger sample should be studied to reduce energy for pulverizing the sample. Additionally, the optimization of the reaction conditions is indispensable for recovering maximum energy as methane with minimum energy for system processing.

Acknowledgement

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5 digested sludge.

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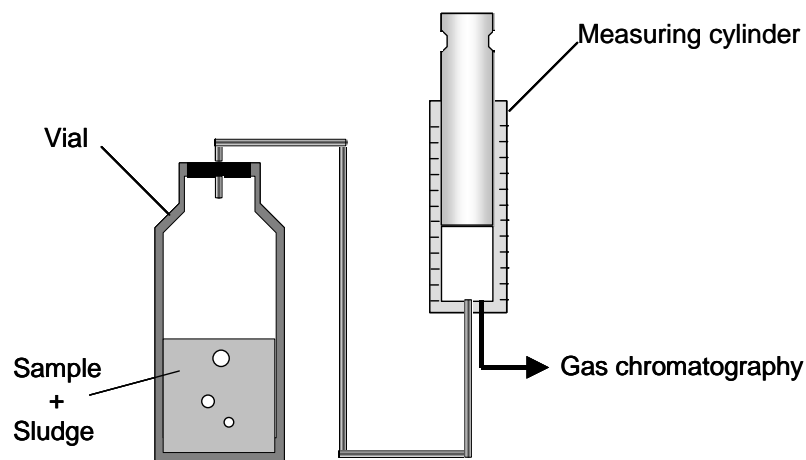


Fig. 1. The apparatus for methane fermentation used in this study.

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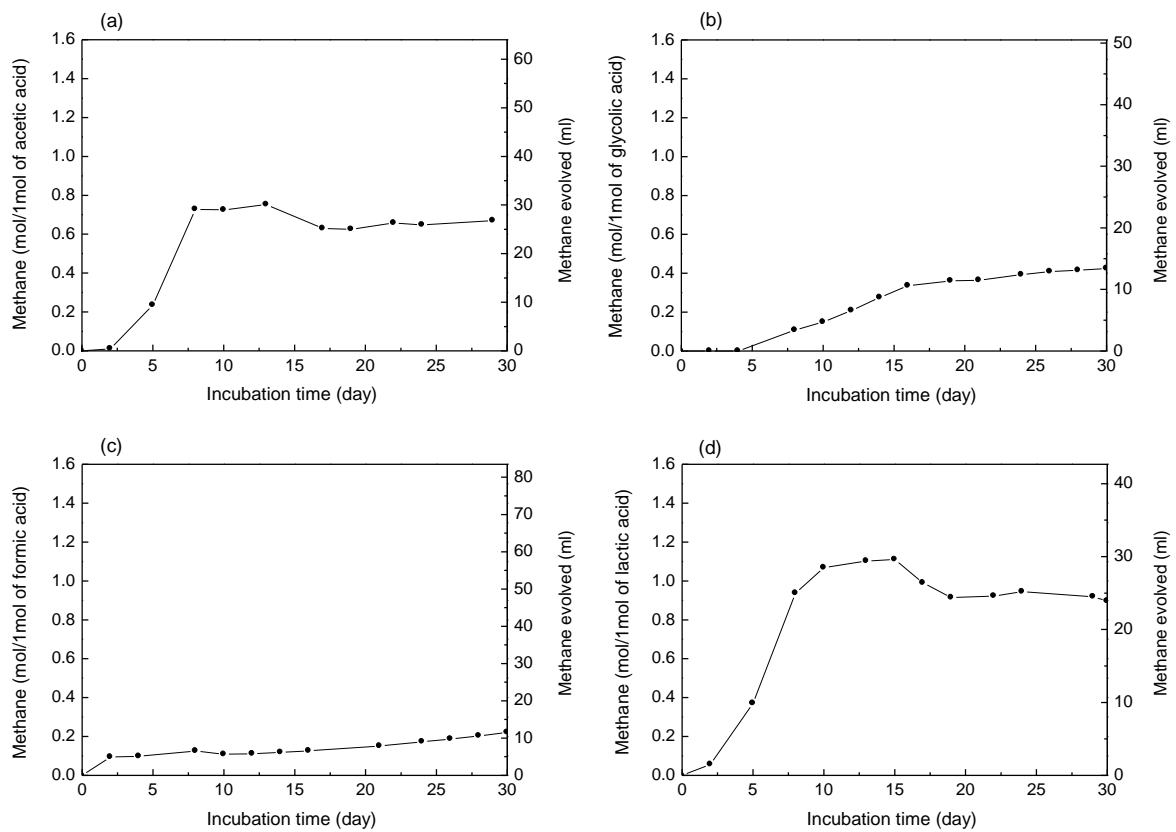


Fig. 2. Comparison of the methane production among various organic acids.
(a) acetic acid; (b) glycolic acid; (c) formic acid; (d) lactic acid.

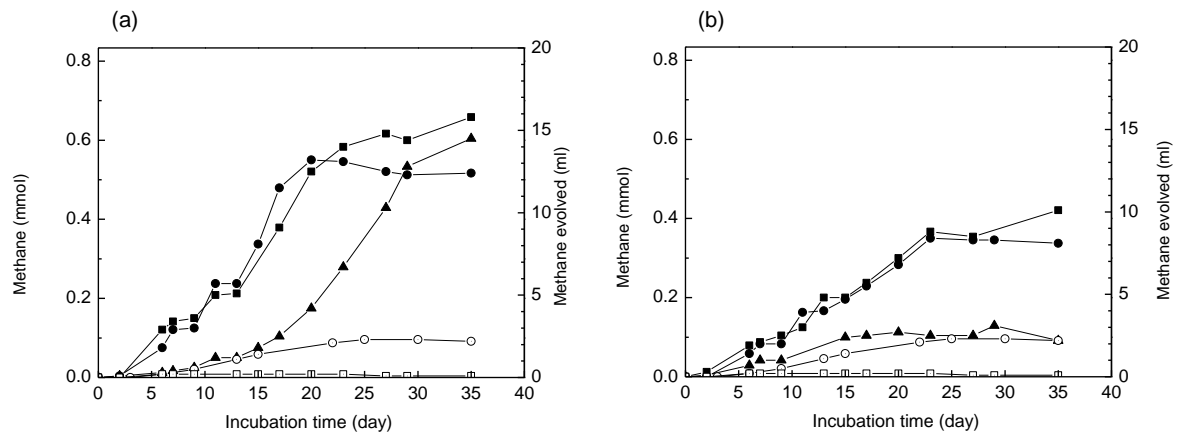


Fig. 3. Methane evolved from the water-soluble portion of beech wood as treated for various reaction times in supercritical water at 380 °C/ 30 MPa (a) and 380 °C/ 100 MPa (b).
filled squares, treatment for 240 s; *filled circles*, treatment for 60 s; *filled triangles*, treatment for 7 s;
open circles, untreated wood; *open squares*, only digested sludge

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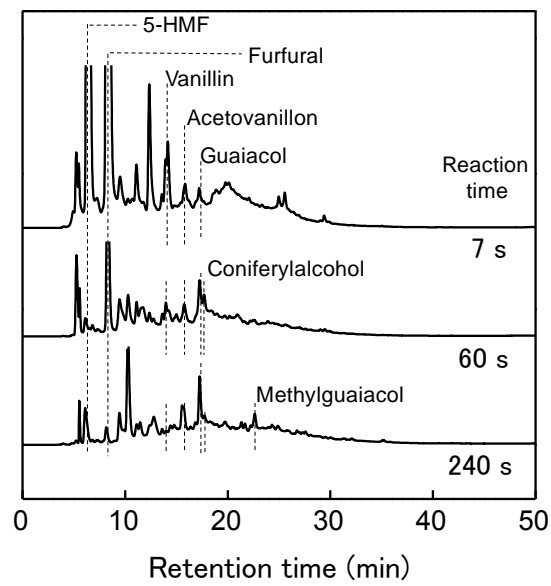


Fig. 4. HPLC chromatograms of the water-soluble portion from beech wood as treated for various reaction times in supercritical water at 380 °C/ 100 MPa .

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Table 1. The sample content with water in reaction vessel in supercritical water treatments.

	Pressure (MPa)	
	30	100
Sample (mg)	79.9	150
Water (ml)	2.61	4.9
Nitrogen (ml)	2.25	0.0

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Table 2. Total content of water-soluble portion and water-insoluble residue from Japanese beech wood as treated in various conditions of supercritical water.

Conditions	Yield (%)													
	Water-soluble portion											Water-insoluble residue		
	Dehydrated products			Fragmented products			Organic acids							
	5-HMF	Furfural	Subtotal	Dihydroxy -acetone	Glycol- aldehyde	Subtotal	Formic acid	Acetic acid	Glycolic acid	Lactic acid	Subtotal			
380°C/ 30MPa/ 7s	0.8	0.8	1.6	8.4	10.0	18.3	0.9	6.6	9.4	4.5	21.4	38.6	79.9	20.1
380°C/ 30MPa/ 60s	0.5	0.6	1.1	6.0	2.4	8.4	0.5	7.5	9.7	5.4	23.1	39.4	72.0	28.0
380°C/ 30MPa/ 240s	0.3	0.9	1.2	4.9	2.9	7.8	0.1	8.6	9.9	3.6	22.2	46.2	77.4	22.6
380°C/ 100MPa/ 7s	3.7	4.4	8.1	1.4	5.6	7.0	0.8	7.9	5.5	2.5	16.7	48.3	80.1	19.9
380°C/ 100MPa/ 60s	0.0	0.7	0.7	1.5	5.8	7.3	0.8	12.3	2.2	1.1	16.5	57.1	81.6	18.4
380°C/ 100MPa/ 240s	0.0	0.1	0.1	1.4	0.0	1.4	0.3	13.5	2.3	0.3	16.5	58.4	76.4	23.6

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Table 3. The percentages of methane evolved from the water-soluble portion of Japanese beech as treated under various conditions of supercritical water to the calculated yields of methane.

Conditions of supercritical water	Percentages to the calculated yield (%)
380 °C/ 30 MPa/ 7 s	105
380 °C/ 30 MPa/ 60 s	97
380 °C/ 30 MPa/ 240 s	117
380 °C/ 100 MPa/ 7 s	37
380 °C/ 100 MPa/ 60 s	92
380 °C/ 100 MPa/ 240 s	96